the Cobalt Development Institute

Dr. A. Persad and Ms. K. Hammerstrom
Integrated Risk Information System (IRIS)
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Cobalt Development Institute and Cobalt Reach Consortium: Data sharing with IRIS

Dear Dr. Persad, dear Ms. Hammerstrom,

the Cobalt Development Institute (CDI) and Cobalt Reach Consortium (CoRC) are conducting cobalt toxicity studies as part of industry's compliance with the EU REACH regulation, as well as to comply with other regulatory needs. These studies are typically guideline compliant and conducted under GLP. We understand that the aim of IRIS is to evaluate information on health effects that may result from exposure to environmental contaminants. Therefore, mainly oral toxicity studies, ideally repeated dose studies, are of interest to IRIS.

In order to ensure transparency as well as harmonisation of data sets used for the evaluation of cobalt toxicity, the CDI and CoRC are willing to share the robust study summaries of the below study list. Please review this list and let us know which of the studies are of interest to the IRIS programme. Please note that most of these studies are part of on-going research programs, and some robust summaries are not yet available (available soon, May 2013).

We can supply the robust study summaries as htm files.

After review of the robust study summaries, please let us know if you would need the complete report of any of the studies. I will then request access to the full report from our industry membership. Please note that, due to ownership of the studies, this is a more involved process.

The same list is being sent to Joyce Donohue at the Office of Water for her review of cobalt toxicity in the context of drinking water.

Please do not hesitate contact me with any questions or additional requests. We look forward to working with the EPA and the IRIS programme,

Sincerely,

Ruth Danzeisen

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Oral repeated dose studies	Co stearate	28-day studies of HPV programme, should already be available to IRIS. An external peer-review, initiated by CDI/CoRC is attached.
	Co borate neodecanoate	
	Co resinate	
	Co acetyl acetonate	
	Co_3O_4	28-day studies, no robust study summaries yet; will be available early May 2013.
	CoS	
	Co powder	

Acute oral studies	$LiCoO_2$	
	CoOOH	
	Co oxalate	Robust study summaries are available
	Co octoate	
	Co isononanoate	
	Co naphthenate	
	Co acetyl acetonate	
	Co propionate	
	Co neodecanoate	
	Co oxalate	
	Co resinate	No robust summaries yet. Will be available in May 2013.
	CoSO4	
	CoO	
	Co powder	
	CoS	
	$LiCoO_2$	
	Co borate propionate	
	Co borate octoate	

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RTI INTERNATIONAL

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June 18, 2010

Dr. Tom Brock
Consulting Toxicologist
The Cobalt Development Institute
Sent by email: tbrock@nipera.org

Dr. Brock:

Attached, please find my reviews of the guideline-compliant OECD 422 reproductive screening studies on four cobalt carboxylic acids in the CD(SD) rat. Based on over 40 years of experience in designing, performing, interpreting, reporting, publishing, and reviewing reproductive, developmental, and endocrine toxicity studies, results from all three of the studies with maternal and offspring toxicity indicated maternal toxicity as the driver for the offspring toxicity, from profound maternal toxicity, leading to profound offspring toxicity at the top doses to lesser maternal toxicity, leading to lesser offspring toxicity at the mid (and sometimes low) doses.

If you have any further comments, questions, or concerns, please do not hesitate to contact me.

Sincerely,

Rochelle W. Tyl, Ph.D., DABT
Distinguished Fellow

Cobalt Neodecanoate

This DuPont study was an OECD 422 repeated dose study with a reproductive/ developmental toxicity screening test (2-week prebreed, 2-week mating, 22-day gestation, and F1 offspring to postnatal day [pnd] 4), with cobalt neudecanoate diluted with corn oil, administered by gavage to Crl:CD(SD) rats (12/sex/dose group) once daily at doses of 5, 15, or 45 mg/kg/day. Hematology, clinical chemistry, gross and histopathologic evaluations, abbreviated neurobehavioral evaluations (FOB, motor activity, and grip strength) were performed on parental males and females.

Results by the Authors

A. No Effects

There were no effects at any dose for:

- F0 (P1) male or female FOB or grip strength
- F0 (P1) female motor activity
- Mating, fertility, precoital interval, or gestational length
- Clinical chemistry parameters in F0 (P1) males or females

B. Effects

1. F0 (P1) Males

At 45 mg/kg/day:

- Clinical signs of toxicity, and decreased body weight, weight gain, feed consumption, and motor activity
- Increased erythropoiesis in spleen and bone marrow

At 15 mg/kg/day:

- Clinical signs of toxicity, and decreased body weight and weight gain

At 5 mg/kg/day:

- Decreased weight gain
- No NOEL but relatively "minor" dose-related signs of toxicity

2. **F0 (P1) Females**

At 45 mg/kg/day:

- Prebreed: decreased weight gain
- During gestation: mortality, decreased body weights and weight gains (at end of gestation), reduced feed consumption and food efficiency, clinical signs of toxicity
- During lactation: mortality, decreased body weights and feed consumption, clinical signs of toxicity
- Total of 11 of 12 dams died at this dose
- Small spleens and stomach ulcer/erosion; tissues from the females at this dose were not evaluated microscopically (p. 36)

At 15 mg/kg/day:

- During gestation: mortality, decreased body weight, weight gain, feed consumption, and food efficiency, clinical signs of toxicity
- Total of 5 of 12 dams died at this dose
- During lactation: reduced body weights
- Reduced thymus weight and increased adrenal weight
- Small spleens and stomach ulcer/erosion
- Gastroenteropathy and lymphoid necrosis and/or atrophy in thymus and spleen
- Minimal vacuolation of renal tubules and minimal microvesiculation of adrenal cortex

At 5 mg/kg/day:

- During lactation: mortality (1 of 12 dams), clinical signs of toxicity
- Gastroenteropathy and lymphoid necrosis and/or atrophy in thymus and spleen
- Minimal vacuolation of renal tubules and minimal microvesiculation of adrenal cortex

3. **F1 Offspring**

At 45 mg/kg/day:

- Reduced uterine implantation sites, corpora lutea, number of pups born, born live, and surviving to pnd 4
- Decreased body weights

At 15 mg/kg/day:

- Decreased body weights (with decreased maternal lactational body weights)

At 5 mg/kg/day:

- Mortality on pnd 0-4
- Decreased body weights

Conclusions by the Study Authors

- No systemic toxicity NOEL; effects in F0 (P1) males and females at all doses
- Reproductive toxicity NOEL; 15 mg/kg/day in females because of reduced ovarian corpora lutea and uterine implantation sites at 45 mg/kg/day
- No offspring toxicity NOEL (effects on offspring at all doses); decreased pup body weights at all doses, mortality at 5 and 45 (but not 15) mg/kg/day

Reviewer's Comments

A. F0 Females

There is clear toxicity to the F0 females – relatively minor during prebreed (only reduced body weights) but profound during gestation and lactation, including mortality during gestation and lactation at 45 mg/kg/day, during gestation only at 15 mg/kg/day, and during lactation only at 5 mg/kg/day. There are also maternal altered organ weights, gross findings (necrosis and/or atrophy in thymus and spleen), and adverse histopathology at all doses.

B. F1 Offspring

At **45 mg/kg/day**, there were reduced ovarian corpora lutea, reduced uterine implantation sites, 11 of 12 maternal deaths, and reduced maternal body weights, so there were maternal effects during and on early *in utero* development. During lactation, there were reduced numbers of total and live born pups on pnd 0 and reduced survival to pnd 4. There were also decreased pup body weights at this dose. At **15 mg/kg/day**, there was only decreased offspring body weights. However, there were maternal gestational deaths (5 of 12), reduced maternal lactational body weights, and adverse histopathology. At **5 mg/kg/day**, in the presence of maternal lactational mortality (1 of 12) and maternal adverse histopathology, there were offspring deaths (pnd 0-4) and reduced pup body weights.

C. General Comments

In this reviewer's professional opinion, the embryofetal and postnatal offspring toxicity at all doses was most likely caused by the profound maternal toxicity (including mortality) at all doses, accompanied by small spleens, stomach ulceration/erosion, reduced thymus weights, and associated histopathology in the spleen, thymus, kidneys, and adrenal cortex in dams at all doses. The maternal effects were clearly extreme enough to cause offspring *in utero* deaths at 45 mg/kg/day, postnatal deaths at 5 and 45 mg/kg/day, and reduced pup weights at all doses.

This reviewer does not agree with the maternal reproductive toxicity NOEL of 15 mg/kg/day by the study report authors. The decreased offspring body weights at all doses and the increased pup mortalities at 5 and 45 mg/kg/day are not "just" developmental toxicity but indicate primary effects on the maternal animals (systemic toxicity to reproductive toxicity, leading to developmental toxicity), resulting in offspring consequences. In this reviewer's opinion, there were no systemic, reproductive, or developmental toxicity NOELs achieved in this study.

Based on the results of this screening study, Cobalt Neodecenoate is NOT a primary reproductive or developmental toxicity. The reproductive and developmental effects observed are most likely due to the maternal systemic toxicity during gestation and/or lactation at all doses evaluated. The doses were all too high and too toxic to the adults and offspring.

Cobalt Naphthenate

This DuPont study was an OECD 422 repeated dose study combined with a reproductive/developmental toxicity screening test (2-week prebreed, 2-week mating, gestation, and F0 dams and F1 offspring terminated on pnd 4 of lactation). Cobalt Naphthenate diluted with corn oil and suspended in mineral spirits was administered once daily by gavage to Crl:CD(SD) rats (12/sex/dose group) at doses of 0, 5, 15, and 50 mg/kg/day. Due to toxicity in the F0 (P1) males, the male dose was dropped to 30 mg/kg/day on test day 16. Ovarian corpora lutea and uterine implantation sites were counted in all females. Histopathologic evaluations of reproductive organs were performed on all high-dose and control males and females. Histologic examination of all other retained tissues was conducted on 5/sex in the high-dose and control groups. Examination of target organs and those with relevant gross lesion was done in the low- and mid-dose groups.

Results by the Authors

A. No Effects

There were no effects at any dose for:

- Abbreviated FOB or grip strength
- Mating and fertility indices, precoital interval, gestational length
- Number of ovarian corpora lutea or uterine implantation sites or sex ratios
- Clinical observations on surviving offspring on pnd 0-4

B. Effects

1. F0 (P1) Males

At 50/30 mg/kg/day:

- Increased mortality
- Clinical signs of toxicity, and decreased body weight, weight gain, feed consumption and food efficiency, and motor activity
- Polycythemia (increased RBC parameters)
- Necropsy and histopathology: decreased absolute and relative liver weights, increased absolute and relative spleen weights, gastroenteropathy, lymphoid

depletion/atrophy in thymus, spleen and/or mandibular lymph nodes, renal tubular degeneration/regeneration, and hepatocellular atrophy

At 15 mg/kg/day:

- Increased clinical signs of toxicity
- Decreased body weights and weight gains, absolute and relative liver weights
- Increased absolute and relative spleen weights, lymphoid depletion/atrophy in thymus, spleen, and/or mandibular lymph nodes, and renal tubular degeneration/ regeneration

At 5 mg/kg/day:

- Increased clinical signs of toxicity and increased absolute and relative spleen weights

2. **F0 (P1) Females**

At 50 mg/kg/day:

- Premating: increased clinical signs of toxicity
- During gestation: increased mortality and clinical signs of toxicity; decreased body weights and weight gains (at end of gestation), reduced feed consumption and food efficiency
- During lactation: increased mortality, clinical signs of toxicity, and actual body weight loss
- Necropsy/histopathology: increased polycythemia, gastroenteropathy, lymphoid depletion/atrophy in thymus, spleen and/or mandibular lymph nodes, renal tubular degeneration/regeneration, renal tubular fatty changes, hepatocellular atrophy, and adrenal cortical necrosis

At 15 mg/kg/day:

- Premating: increased clinical signs of toxicity
- During gestation: increased mortality and clinical signs of toxicity; decreased body weight gains, feed consumption, and food efficiency
- During lactation: increased mortality, clinical signs of toxicity
- Necropsy/histopathology: gastroenteropathy, lymphoid depletion/atrophy in thymus, spleen and/or mandibular lymph nodes, renal tubular fatty changes, hepatocellular

atrophy, and adrenal cortical necrosis, renal tubular degeneration/regeneration, renal tubular fatty changes, and hepatocellular atrophy

3. **F1 Offspring**

At 50 mg/kg/day:

- Decreased number of pups born, total and liveborn, and reduced survival to pnd 4
- Decreased body weights

At 15 mg/kg/day:

- Decreased number of pups born, total and liveborn, and reduced survival to pnd 4

At 5 mg/kg/day:

- Decreased survival during pnd 0-4 (lactation)

Conclusions by the Study Authors

The authors concluded that, under the conditions of the study, NOELs for systemic, reproductive, and developmental toxicity were not achieved due to parental and offspring toxicity at all doses, including the lowest dose of 5 mg/kg/day

Reviewer's Comments

This reviewer concurs with the lack of NOELs for systemic, reproductive, and developmental toxicity. She was impressed with the concordance of the degree of maternal toxicity across doses with the degree of offspring toxicity.

At **50 mg/kg/day**, there was increased maternal mortality during gestation and lactation and decreased body weights and feed consumption, increased clinical signs of toxicity, and profound histopathology. The offspring exhibited reduced litter sizes at birth (both total and live born), reduced survival on pnd 0-4, and reduced body weights. At **15 mg/kg/day**, there was increased maternal mortality during gestation and lactation, increased clinical signs of toxicity, reduced body weights, weight gain, and feed consumption during gestation, and profound histopathology. The offspring exhibited reduced litter sizes (both total and live born) and reduced survival to pnd 4. At **5/mg/kg**, there were increased maternal clinical signs of toxicity and, during lactation, increased mortality and clinical signs of toxicity, and limited adverse

histopathology (gut and liver). The offspring exhibited decreased survival during pnd 0-4 (lactation).

The most likely (conservative and appropriate) interpretation is that the maternal systemic toxicity caused the subsequent reproductive toxicity and offspring developmental toxicity. Although all of the groups exhibited both parental and offspring toxicity, the degree of parental (especially maternal) toxicity presaged and predicted the offspring toxicity.

It is clear that Cobalt Naphthenate is not a primary reproductive or developmental toxicant; parental systemic toxicity drove the other "toxicities." It is also clear (to this reviewer) that all the doses were too high to determine NOELs for systemic, reproductive, or developmental toxicity.

Cobalt (II) Stearate

This DuPont study was an OECD 422 repeated dose study combined with a reproductive/developmental toxicity screening test (2-week prebreed, 2-week mating, gestation, and F0 dams and F1 offspring terminated on pnd 4 of lactation). Cobalt Stearate diluted with 0.1% Tween -80 and suspended in 0.5% aqueous methylcellulose (5 ml/kg) was administered once daily by gavage to Crl:CD(SD) rats (12/sex/dose group) at doses of 0, 5, 15, and 40 mg/kg/day for males and at 5, 15, or 100 mg/kg/day for females. Ovarian corpora lutea and uterine implantation sites were counted in all females. Clinical chemistry and hematology evaluations were done at the end of the premating period and at terminal sacrifice. FOB, motor activity, and grip strength were assessed once during "pretest" (quarantine) and prior to cohabitation. Histopathologic evaluation of reproductive organs was performed on all high-dose and control males and females. Histologic examination of all other retained tissues was conducted on 5/sex in the high-dose and control groups. Examination of target organs and those with relevant gross lesion was done in the low- and mid-dose groups.

Results by the Authors

A. No Effects

1. F0 (P1) Males

There were no effects at any dose for:

- Body weights, feed consumption, food efficiency, mortality, clinical signs, FOB, motor activity, grip strength, hematology, coagulation, or clinical chemistry; there were no adverse effects at any dose

2. F0 (P1) Females

- Body weights, feed consumption, food efficiency, mortality, or clinical signs during premating at 5, 15, or 100 mg/kg/day
- Body weights, feed consumption, food efficiency, mortality, or clinical signs at 5 mg/kg/day during gestation and lactation (there were effects at 15 and 100 mg/kg/day during these periods)

3. Reproduction

No effects on mating or fertility indices, precoital interval, gestational length, number of ovarian corpora lutea, number of uterine implantation sites, or "implantation efficiency" (the obverse of preimplantation loss).

4. Development

No effects on litter clinical observations, mean pup weights on pnd 0-4 at any dose; no effects on number of pups born, sex ratio of pups, or pup survival indices during the lactation period at 5 and 15 mg/kg/day (there were developmental effects at 100 mg/kg/day).

5. Gross and Histopathology

No effects on organ weights or gross pathology in either F0 (P1) males or females at any dose and no microscopic pathology in F0 (P1) males at any dose (there were effects in females).

B. Effects

1. F0 (P1) Females

At 100 mg/kg/day:

- During gestation: mortality (10 of 12 dams died), clinical signs of toxicity, decreased body weights and weight gains, reduced feed consumption and food efficiency
- During lactation: decreased body weights and feed consumption
- Microscopic pathology: lymphoid necrosis/atrophy, enteropathy and renal tubular vacuolation, adrenal gland cortical microvesiculation and necrosis

At 15 mg/kg/day:

- During gestation: clinical signs of toxicity and reduced feed consumption
- During lactation: reduced feed consumption
- Increased incidences of microscopic pathology, including lymphoid necrosis/atrophy and adrenal gland cortical vesiculation or necrosis

At 5 mg/kg/day:

- No adult F0 (P1) female effects

2. **F1 Offspring**

At 100 mg/kg/day:

- Decreased numbers of live born pups on pnd 0 (only 1 litter)

At 15 mg/kg/day:

- Decreased numbers of live born pups on pnd 0 and 4

At 5 mg/kg/day:

- No effects on *in utero* or postnatal development

Conclusions by the Study Authors

The authors concluded that, under the conditions of the study, NOELs for systemic and reproductive toxicity for F0 (P1) females "was considered 5.0 mg/kg/day" (p. 12) because of adverse effects on the adult females and decreased numbers of live born pups at 15 and 100 mg/kg/day. The NOEL for F0 (P1) males "was 40 mg/kg/day, the highest dose tested" (p. 12).

Reviewer's Comments

The text indicates no effects on the "numbers of pups born, sex ratio, and survival indices during the lactation period in F1 litters for 5 and 15 mg/kg/day" (p. 12). However, elsewhere they report that there were reductions in the "number of pups born alive and number of pups on day 4 of lactation" at 15 mg/kg/day. The conclusion and NOELs are correct, but the summary text is not.

In this study, the F0 (P1) males were apparently unaffected at 5 through 40 mg/kg/day. The F0 (P1) females were adversely affected at 15 and 100 mg/kg/day. Their F1 offspring were also affected at these doses. The greater maternal toxicity at 100 mg/kg/day, included mortality, clinical toxicity, decreased body weights and weight gains, decreased feed consumption, and adverse histopathology (including lymphoid and adrenal gland adverse histopathology, enteropathy, and renal tubular damage) and was mirrored by (and likely caused) the greater offspring toxicity at this dose (decreased numbers of live born pups; only one litter survived at this dose).

At 15 mg/kg/day, maternal toxicity was lesser (clinical signs of toxicity, reduced feed consumption, and lymphoid and adrenal gland histopathology), and the offspring effects were lesser: decreased number of pups on pnd 0 and 4 (there was no effect on viability index; the

reduced numbers on pnd 0 were the reason for the reduced numbers [same as on pnd 0] on pnd 4). The most appropriate (likely and conservative) conclusion is that the profound maternal toxicity at 100 mg/kg/day resulted in the profound effects on the offspring (only one litter), and that the lesser maternal toxicity at 15 mg/kg/day resulted in the lesser offspring toxicity (reduced litter sizes).

Cobalt (II) Stearate is not a primary reproductive or developmental toxicant. It is clear to this reviewer that the continuing and dose-related adult maternal toxicity at 15 and 100 mg/kg/day was responsible for the developmental offspring toxicity also at 15 and 100 mg/kg/day.

Cobalt Borate Neodecanoate

This DuPont study was an OECD 422 repeated dose study with a reproductive/ developmental toxicity screening test (2-week prebreed, 2-week mating, 22-day gestation, and F1 offspring to postnatal day [pnd] 4), with cobalt borate neodecanoate diluted with Mazola® corn oil, and administered by gavage to Crl:CD(SD) rats (12/sex/dose group) once daily at doses of 0, 0.5, 1.5, or 5.0 mg/kg/day. Hematology, clinical chemistry, gross and histopathologic evaluations, and abbreviated neurobehavioral evaluations (FOB, motor activity, and grip strength) were performed on parental males and females.

Results and Conclusions

The authors of the study report concluded that the NOEL "was considered 1.5 mg/kg/day" due to a statistically significant increased sex ratio (more males) in the F1 offspring at 5.0 mg/kg/day, based on 11 litters out of 12 mating pairs. The controls had a sex ratio of 39.39% males (more females and outside the historical control range), and the 5.0 mg/kg/day group had a pup sex ratio of 64.96% (more males, and again outside the historical control range of 45-59% males). However (p. 29), there is no evidence of increased preimplantation loss (the difference between the number of ovarian corpora lutea as a measure of eggs ovulated) or of postimplantation loss at 5.0 mg/kg/day (numbers of uterine implantation sites as a measure of conceptuses which implanted), expected if the female offspring preferentially died, and no evidence of reduced litter size (again expected if female offspring were lost. There is also no evidence of reduced pup body weights per litter at any group, including the 5.0 mg/kg/day group on pnd 0 or 4. However, the report did not present pup body weights by sex by litter, so we do not know whether the "normal" body weight at 5.0 mg/kg/day was accurate or from the excess males (males are heavier) at the top dose; there might have been a body weight effect.

I do not consider skewed sex ratios as a "toxic" endpoint, and there are no endpoints (e.g., body weights, feed consumption, clinical signs, behavioral tests, clinical chemistry, organ weights, histopathology, etc.) which indicate any toxicity to the adults or offspring. I believe that this study did NOT identify a dose with toxicity to either the adults or the offspring. All things considered, I recommend that the study be repeated at higher doses (with a dose range-finding study run first to identify the appropriate doses for the subsequent OECD 422 study.